

Claims:

1. A method for producing a mutant gene encoding a functional gene product, the method comprising:
  - (a) introducing one or more mutations into a gene to form a mutated gene;
  - 5 (b) providing the mutated gene to a host microorganism;
  - (c) culturing the host microorganism containing the mutated gene under conditions which allow expression of the mutant gene product; and
  - (d) selecting a host microorganism capable of producing a functional gene product from the mutated gene.
- 10 2. The method according to claim 1 comprising:
  - (a) introducing one or mutations into a gene to form a plurality of mutated genes;
  - (b) providing the mutated genes to host microorganisms;
  - (c) culturing the host microorganisms containing the mutated genes under conditions which allow expression of the mutant gene products; and
  - 15 (d) selecting host microorganisms capable of producing a functional gene product from a mutated gene.
3. The method according to claim 2 further comprising:
  - (e) obtaining a combined pool of mutated genes encoding functional gene products from the microorganisms in step (d) and repeating steps (a) to (d) to form a library of
  - 20 microorganisms containing a plurality of mutant genes capable of being expressed and producing functional gene products.
4. The method according to claim 3 further comprising:
  - (f) screening the library of microorganisms to obtain a mutant gene capable of expressing a mutant gene product.
- 25 5. The method according to any one of claims 1 to 4 wherein the mutant gene product has an altered or desired activity, function, or characteristic compared with the native gene product.
6. The method according to any one of claims 1 to 5 wherein step (a) is carried out by mis-incorporation mutagenesis using polymerase chain reaction (PCR) or gene
- 30 shuffling.
7. The method according to any one of claims 1 to 6 wherein step (b) is carried out by ligating the mutant gene into a vector and transforming a bacterium with the vector.

8. The method according to claim 7 wherein the vector is a plasmid or virus and the bacterium *Escherichia coli*.
9. The method according to any one of claims 1 to 8 wherein the host microorganisms are cultured under conditions in which the native gene would be expected to be expressed in the particular host microorganism.
10. The method according to claim 9 wherein host microorganisms are cultured in a liquid medium.
11. The method according to any one of claims 1 to 10 wherein the culture conditions result in any host microorganism capable of expressing a functional gene having a detectable characteristic.
12. The method according to claim 11 wherein the detectable characteristic is a selectable phenotype selected from the group consisting of colour, size, shape, fluorescence, dependency on a supplied metabolite for growth, enzymatic activity such as enzymatic conversion of a supplied substrate, and combinations thereof.
13. The method according to claim 12 wherein the gene encodes an enzyme that can form a fluorometric or chromogenic phenotype or character in a microorganism expressing the gene.
14. The method according to any one of claims 1 to 13 wherein the gene product is an enzyme.
15. The method according to claim 14 wherein the selecting is carried out by sorting by flow-cytometry and the bacteria selected by changes in their spectral or fluorescence characteristics due to action of the enzyme on the substrate.
16. The method according to claim 14 or 15 wherein the enzyme is capable of acting on an X-sugar or a fluorescein-linked sugar substrate.
17. The method according to claim 16 wherein the substrate is an indoxyl-linked compound.
18. The method according to claim 17 wherein the enzyme acting on an indoxyl-linked substrate is selected from the group consisting of glycosyl hydrolases, cellulases, beta-glucosidases, beta-galactosidases, mannosidases, xylanases, and beta-xylosidases.
19. The method according to claim 18 wherein the enzyme is capable of acting on 5-Bromo-4-chloro-3-indolyl -D-galactopyranoside which forms a chromogen upon enzymatic hydrolysis.

20. The method according to any one of claims 1 to 18 wherein the gene product substrate is retained on, or within the cell, in liquid culture.
21. The method according to any one of claims 1 to 20 wherein the host microorganism capable of producing a functional gene product from the mutated gene is selected by
- 5 22. A mutant gene capable of producing a mutant gene product produced by the method according to any one of claims 1 to 21.
23. A method for producing a mutant gene encoding a functional enzyme, the method comprising:
- 10 (a) introducing one or more mutations into a gene encoding the enzyme to form a plurality of mutated genes;
- (b) incorporating the mutated genes into vectors;
- (c) transforming host microorganisms with the vectors;
- (d) culturing the host microorganisms containing the mutated genes under conditions which would allow expression of the corresponding native gene to produce the native
- 15 enzyme such that the microorganisms express the mutant genes;
- (e) providing a substrate upon which the enzyme can act to produce a selectable phenotype in any host bacterium producing a functional enzyme; and
- (f) selecting host microorganisms capable of producing a functional gene product from the mutated gene by sorting bacteria having the selectable phenotype.
- 20 24. The method according to claim 23 further comprising:
- (g) obtaining a pool of mutated genes from the microorganisms in step (f) and repeating steps (a) to (f) to form a library of microorganisms containing a plurality of multiply-mutant genes capable of being expressed and producing functional enzymes.
- 25 25. The method according to claim 24 further comprising:
- (h) screening the library of microorganisms to obtain a mutant gene capable of expressing an enzyme with an altered or desired activity, function, or characteristic.
26. The method according to any one of claims 23 to 25 wherein the selecting is carried out by sorting by flow-cytometry and the bacteria selected by changes in their
- 30 spectral or fluorescence characteristics due to action of the functional enzyme on the substrate.
27. A mutant gene capable of producing a mutant gene product produced by the method according to any one of claims 23 to 26.